EXPLOITATION OF MICROALGAE FOR BIOPRODUCTS PRODUCTION USING DAIRY EFFLUENT

SYNOPSIS SUBMITTED

By

Nisha Dhillon

Registration No - DD20205010001

Under the guidance of
Dr. Sanjay Gupta
Professor and Head
Department of Biosciences

For Ph.D. Degree in Biochemistry

Swami Rama Himalayan University
Swami Ram Nagar, Jolly Grant Dehradun,
Uttarakhand
India

DECLARATION

I hereby declare that the research synopsis entitled **"EXPLOITATION OF MICROALGAE FOR BIOPRODUCTS PRODUCTION USING DAIRY EFFLUENT''**is my own original work and it has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles.

Signature of Ph.D. Scholar
Name of Ph.D. Scholar: Nisha Dhillon
Department: Deptt. of Biosciences

Signature of Supervisor
Name of Supervisor: Dr.Sanjay Gupta
Designation: Professor & Head
Department: Deptt. of Biosciences

Signature of Co-Supervisor:
Name of Supervisor: Dr. Vivek Kumar
Designation: Associate professor
Department: Department of Biosciences

Table of Contents

S.NO	CONTENTS	PAGE NO.
1	Introduction	1
2	Review of Literature	4
3	Aim & Objectives	6
4	Materials and Methods	7
5	Expected Outcomes	8
6	Timelines of the Proposed Research Work	8
7	References	10

EXPLOITATION OF MICROALGAE FOR BIO-PRODUCTS PRODUCTION USING DAIRY EFFLUENT

INTRODUCTION:

In the current scenario, increasing globalization leads to the rapid industrialization in order to fulfil the requirement of food and feed. Recently, there have been different types of industries which had developed and guzzle lots of water and resulted in the production of life threatening or toxic compounds whichare released into the environment, which could be a cause of different types of hazardous diseases. Though the industries are an essential component of economy, but rapid industrialization has become a matter of major concern due to their deteriorating activity on the environment. Different types of industries produce different types of waste water, among these the dairy industry is one of them and produces huge amount of waste water in the form of strong pollutants.[1] The dairy industry is a major user of water and generator of huge amount of wastewater.

Dairy industries have played a significant role in Indian economy and provide great job prospects, further; this industry is growing day by day. Despite being one of the biggest contributors of wastewater, dairy industries have a positive impact on a nation's economic growth. According to FAO 2020, India is the largest milk producer in the world with 22% of global milk production followed by USA, Pakistan. The dairy industries produce enormous quantities of wastewater and globally it has a strong environmental footprint. They utilize more water than any other agricultural industry and it has been estimated that for every litre of milk produced uses approximately three litres of water.[2] Dairy products, especially milk has recorded an increase in production and consumption. Globally, the consumption of dairy products is predicted to increase by 13.7% by 2023.[3] if this prediction becomes true, and then the wastewater generated is expected to increase significantly.

Dairy industry primarily produces liquid effluent during the processing of different useful products from whole milk like buttermilk, condensed cream, sweet whey, curd, cheese, butter, and ice cream, etc. The distinguishing features of dairy wastewater might be varying according to the formation of final products. Generally, dairy effluents containing high concentration of lipids such as triacylgycerides, phospholipids, saturated and unsaturated fatty acids, carbohydrates such as; lactose, glucose and galactose etc. The dairy waste water also

contains two major soluble proteins casein and whey protein etc., resulting in high BOD and COD levels.[4,5] Its high biological oxygen demands deplete the dissolved oxygen content and create anaerobic conditions very rapidly under the aquatic system. Dairy effluent contains various inorganic and organic compounds of nitrogen and phosphorus, present in higher concentration and responsible for the alkalinity of the dairy waste and other than it also contains trace amount of some mono-valent and bi-valent cations like Na, Mg, Ca, Fe, K, Ni, Mn etc. Use of detergents and chlorine in the dairy industry further deteriorates the quality of effluent.[6, 7]

Dairy effluents are biologically active because of the presence of high organic load and when it is discharged either untreated or semi-treated into water streams leads to deficiency in oxygen level. Hence, the proper treatment before the disposal of dairy waste effluent has become the major problem for the vitality of the dairy industry and environment.[8] Various attempts have been already made to solve this problem by reusing the nutrients rich dairy waste with microorganisms to metabolize organic matter.

There are different technologies which can be used for the treatment of dairy waste before being released into the natural habitat or surroundings.[9] There are several physico-chemical, and biological methods used for the bioremediation of dairy waste. Among these methods, biological method is ecofriendly, cost-effective and potential to mitigate the contaminants. This approach uses various micro-organisms like bacteria, fungi, blue green algae (microalgae) for the dairy waste water treatment.[10, 11, 12]

Dairy wastewater has high levels of phosphorus, nitrogen, and organic matters thus, the nutrient rich dairy waste effluent proved to be a marvellous medium for the growth of microalgae. Earlier researches suggested that cultivation of microalgae for the production of lipid components from dairy waste, had provide better results than any other wastewater used as microalgae cultivation medium.[13] Many microalgae cultures displayed high growth on the diluted effluents from dairy.[14] Microalgae represents promising biological system for treating dairy wastewater due to their metabolic flexibility, perform as photoautotrophic, mixotrophic, or heterotrophic metabolic activity. Moreover, the microalgae have the ability to use solar energy for its photosynthetic activity and combine water with carbon dioxide in order to make their food autotrophically, further; it converts the environmental carbon to oxygen.

Due to this ability of microalgae, they have been used to remediate the dairy waste as the nutrient sources for their growth, and to reduce the concentrations of nitrogen, phosphorus and other inorganic substances. It has been reported that the microalgae is the better source for manufacturing of bio-fuel due of its elevated photorespiration activity, and rapidly growing biomass activity.[15, 16] Micro algal biomass have been used for production of biological compounds like single cell protein, bio-ethanol, biogas, hydrogen gas and lutein or astaxanthin[17] and can also be used as bio fertilizers.[18, 19, 20, 21] The microalgal strain which contain large amount of fatty acids, like polar and non-polar or neutral lipids, and also flourishing in the native domain of dairy waste effluent have yet to be used for bio-fuel production.[22] Micro algal feedstock is one of the best medium for bio-fuel production and as a replacement source of fossil fuel and also proved beneficial to eradicate greenhouse gas emissions.[23]

Many microalgal species have been analyzed for their fatty acid compositions, approx 20-50 % of dry biomass including components of lipids. Some microalgae such as species of *Nannochloropsis*, *Neochloris*, *Chlorella*, *Dunaliella*, *Crypthecodinium*, *Cylindrotheca*, *Isochrysis*, *Nannochloris*, *Nitzschia*, *Schizochytrium*, *Phaeodactylum*, *Porphyridium*, and *Tetraselmis* have been recognized for containing certain classes of polar and non-polar lipids. Nonpolar or neutral lipids like sterols, free fatty acids acylglycerols, etc. and polar lipids like glycosylglycerides, phosphoglycerides, and sphingolipids have been reported in microalgae.[24, 25]

There are very few studies which presented the complete characterization of lipids of a particular micro alga. Due to the large variation among the species of microalgae and their cultivation conditions, it is often difficult to compare the results for a comprehensive biochemical profiling of bio molecules such as lipid, protein, and carbohydrates etc. present in all microalgae species. Because of this reason, there is still a need to fully characterize the various bio molecule components within one alga species and helps in demonstrating the appropriate analytical method which can be employed for further use. The complete characterization of lipid in microalgae is also essential for the selection of good species, so that the production of lipids can be utilized as bio-fuels and other high-value compounds in the field of food, pharmacy.[26]

There are various strains of microalgae (*Chlamydomonas, Chlorella* sp. etc.) which have been employed as an experimental design to examine its anabolic and catabolic activities and functions. Genomes of *Chlamydomonas reinhardtii* have been sequenced and its molecular and genomic tools is also available.[27, 28] It can also accumulate considerable value of triacylglycerols under salt stress or nitrogen deprived conditions.[29, 30, 31, 32, 33, 34, 35]

Micro algal species collected from the dairy waste indicated great prospective for low cost cultivation and producing lipid from dairy waste, without modifying the procedure. It was reported that the *Chlorella* sp. isolated from the dairy waste water showed good biomass production when grown on dairy effluent, reduced its chemical oxygen demand, and nutrients.[14] This microalga was identified by using 18S rDNA sequencing, and the DNA sequences were analyzed via BLAST representing the high homology with *Chlorella* sp. ArMoo29B (98% homology).

REVIEW OF LITERATURE:

Patel et al. documented about whey utilization for getting biomass of algae to enhance dairy industry carbon footprints in addition to remove contaminants and also getting energy gain. Microalgae *Chlorella protothecoides* displayed good growth in a pre-treated whey solution. Since the use of microalgae approach lonesome was not sufficient for satisfactory removal of pollutants, therefore using hydrolysis and flocculation-struvite methods, the treated whey with 25:75 dilutions exhibited sufficient pollutants remedy by designed biochemical approach. Using this approach, approximately up to 99% or 91–100% lessening of inorganic and organic contaminants was attained after nine days of cultivation of microalgae. Results showed that using dairy wastewater resulted in enhanced carbon footprints and ecological benefits along with energy and carbon recovery.[5]

Brar et al. investigated the efficacy of microalgal species for bioremediation of dairy waste water and bioenergy production from its nutrients. The amount of N and P along with other nutrients present in dairy waste water can reduce the cost of biomass production by dismissing the outside nutrient supplementation. The superior biomass production of *Chlorella pyrenoidosa, Anabaena ambigua* and *Scenedesmus abundans* was observed in 3:1 dairy waste water over control. The microalgal species have efficiently decreased the COD, BOD, NO_3^-, and PO_4^{-3}. The biochemical characterization of microalgae showed the good

amount of lipid and protein production after cultivating 25th day in 3:1 ratio of dairy waste water.[39] In the similar study, conducted by Young et al. reported that freshwater green microalgae *Chlorella* sp., UMACC344 was produced higher lipid amount, which could be utilized as potential biofuel.[40]

In another study, Choi et al. isolated *Chlorella* sp. from dairy wastewater. Cultivation of *Chlorella* sp. on this dairy waste water resulted in higher algal biomass production along with good amount of lipid production. The microalga *Chlorella* sp., displayed good growth on dairy waste water having high BOD and COD, trace elements and other nutrients, owing to its ample acclimatization on to the waste water.[14]

Rani et al. studied that the dairy effluent was efficient for the growth of *Chlorella vulgaris* and *Anabaena ambigua*, with the significant lipid, carbohydrates, protein productivity. Both the cultures were optimized in BG11 media. Lipid and carbohydrate content was found high in *Chlorella vulgaris* when compared to *Anabaena ambigua*, which has high protein content. Ammonium nitrates and phosphates were almost completely removed by both algae. A significant decrease in both COD and BOD, and the current study, resulted that the ability of algae to remove pollutants.[36]

Saud concluded an assessment of an industrial dairy wastewater and stated that *Spirulina* sp. viable for biological treatment as it showed the reduction in nutrients concentrations from wastewater such as, nitrate, phosphorus and sulphate. It also reduces total hardness, alkalinity, chloride, COD, TDS, Ca and Mg hardness. The recorded ability of that algae on removing nitrate 80 %, active phosphorus 72% and sulphate 61% While total hardness 56%, alkalinity was 71%, chloride 56%, C.O.D 77% T.D.S 54%, Ca and Mg hardness 59% during the tenth day of treatment.[7]

Sukumaran reported that *Chlorococcum* sp. RAP13 grown in dairy effluent generated great amount of biological mass and lipids, particularly under heterotrophic growth conditions with supplement of biodiesel industry waste glycerol. The micro algal cells showed growth characteristics differences, lipid contents and fatty acid profile. This difference was based on modifications in carbons sources and the growth conditions. Lipid production and biomass increased expressively under heterotrophic cultural conditions. Higher amount of lipid production by the micro algae makes it a suitable candidate for biodiesel production.

Moreover, use of dairy effluent as a source of energy and carbon by micro algae improved its quality in terms of BOD and COD.[38]

Kothari et al. exhibited that dairy business wastewater could be utilized as source of nutrients for production of algal biomass. They reported that growing *Chlamydomonas polypyrenoideum* on dairy wastewater resulted in considerable lessening of nitrogen (90%) and phosphate (96%) on 10^{th} and 15^{th} day respectively. They found that nitrogen and phosphate were the chief reason of any water body resulting in eutrophication. The resultant biomass of microalgae after treatment of 10^{th} day exhibited 42% (w/w) lipid contents, therefore, considered being a probable biological oil source, which was analogous to the bio-oil extracted from other natural resources as obvious from FTIR results analysis. These outcomes proposed the possible usage of microalgae for biodiesel production as well as phyco-remediation of the dairy wastewater.[37]

Msanne et al. reported that green alga *Chlamydomonas reinhardtii* gathers noteworthy amount of triacylglycerols (TAGs) under N limitation or salt stress in culture medium encompassing acetate. A noticeable reduction in protein and chlorophyll contents was also detected, including lessening in ribosomal polypeptides and some vital enzymes for carbon dioxide assimilation, such as ribulose-1, 5-bisphosphate carboxylase/oxygenase. These results proposed that throughput of N rich complexes such as proteins may supply energy and carbon for biosynthesis of triacylglycerols in the nutrients run-down cells.[21]

AIMS AND OBJECTIVES:

The aim of this study is to manifest that dairy wastewater could be utilized as a possible large-scale cultivation medium for growing the micro algal consortia. This will be effectively used for the removal of pollutants from dairy waste water and it will be used as an efficient biodegradative agent as well as sources for the production of energy rich bioproducts, in future. Employment of microalgal species for different wastewaters bioremediation has been widely studied. Bioremediation of dairy effluent using potential autochthonous microlagal species is not a regular feature. Further, bioremediation of dairy waste water along with the concurrent production of lipids is not a recurrent subject. Therefore, this study has been designed to bioremediate the dairy waste water using microalgae along with production of

lipids, which could be exploited for the bio-suitability (biofuel, food or pharma biomolecules etc.).

Therefore, keeping the above in view and also beneficial aspects of microalgae use in dairy industry, the present study has been planned with the following objectives;

OBJECTIVES:

1. To isolate different autochthonous microalgae species from dairy waste water.
2. Microalgal sp. will be screened for dairy effluent remediation, higher biomass and lipid production.
3. Identification of higher lipid producing microalgal species.
4. Microalgal lipids will be characterized for its bio-suitability.

MATERIALS AND METHODS:

1. To isolate different autochthonous microalgae species from dairy waste water.

- Different microalgal species will be isolated from dairy waste water using standard protocol described by Andersen[46] and Morando-Grijalva et al.[41]

2. Microalgal species will be screened for dairy effluent remediation, higher biomass and lipid production.

- Dairy wastewater (DWW) is having high BOD, COD, fat, grease and oil along with soluble solids, nitrogen and phosphorous (12). These nutrients act as a good source of nutrients for microalgae, but higher BOD, COD and nutrients poses environmental nuisance and algal bloom issues.
- Microalgae will be grown on dairy wastewater to study the following parameters.

- Bioremediation of DWW, lowering of BOD and COD, nitrogen and phosphorous content, will be carried out as previously mentioned.[42]
- Different concentrations of DWW will be optimized for microalgal higher biomass and lipid production.[43, 44]

3. **Identification of higher lipid producing microalgal species.**

- Higher lipid producing microalgal species will be characterized and identified by studying their morphological features using compound microscope.
- After morphological characterization of microalgae, the phylogenetic analysis will be carried out by employing 18S rRNA sequence analysis as described by Liu et al.[45]

4. **Microalgal lipids will be characterized for its bio suitability.**

- Microalgae lipids will be extracted as mentioned by Hena et al.[43] and characterized for composition of fatty acids in total lipids, hydrocarbons, and sterols by GC-MS[26] This information will let us know about type of lipids to be exploited as future biomolecule.

EXPECTED OUTCOME:

1. Identification of potential microalgae as bioremediator of dairy wastewater.
2. Reveal the role of single microalgae or consortia on dairy waste water remediation.
3. Information generated about type and amount of lipid production by microalgae will be useful for researchers, students and industries.
4. Publications and patents

TIMELINES OF THE PROPOSED RESEARCH WORK:

First Year:

- Collection of sample (dairy waste water) from Dairy industries.
- Isolation of different autochthonous microalgae species from dairy waste water using standard protocol (Andersen and Morando-Grijalva et al.).[41,46]
- Microalgal species will be screened for dairy effluent remediation, higher biomass and lipid production.

Second Year:

- Identification of higher lipid producing microalgal species will be characterized and Identified by studying their morphological features using compound microscope.
- The phylogenetic analysis will be carried out by employing 18S rRNA sequence analysis as described by Liu et al.[45]

Third Year:

- Microalgae lipids will be extracted as mentioned by Hena et al.[43]
- Profiling of fatty acids in total lipids, hydrocarbons, and sterols by GC-MS and Glyco-phospholipids by LCMS.[26]

SIGNIFICANCE OF THE STUDY:

The utilization of nutrients from dairy wastewater by autochthonous microalgae and remediating it so that it can be discharged into natural streams, since the wastewater is no more environmental issue. The compositional information generated will be a beneficial reference for future study on micro algal species selection and cultivation optimization, as well as for the production of value-added co-products from microalgae for beneficial biomolecules.

RFERENCES:

1. Swain P, Tiwari A, Pandey A. Enhanced lipid production in Tetraselmis sp. by two stage process optimization using simulated dairy wastewater as feedstock. Biomass and Bioenergy. 2020 Aug 1;139:105643.
2. Porwal HJ, Mane AV, Velhal SG. Biodegradation of dairy effluent by using microbial isolates obtained from activated sludge. Water Resources and Industry. 2015 Mar 1;9:1-5.
3. Choi HJ. Dairy wastewater treatment using microalgae for potential biodiesel application. Environmental engineering research. 2016;21(4):393-400.
4. Mehrotra R, Trivedi A, Mazumdar SK. Study on characterisation of indian dairy wastewater. International Journal of Engineering Applied Sciences and Technology. 2016;1(11):77-88.
5. Patel AK, Joun J, Sim SJ. A sustainable mixotrophic microalgae cultivation from dairy wastes for carbon credit, bioremediation and lucrative biofuels. Bioresource technology. 2020 Oct 1;313:1236-81.
6. Kushwaha JP, Srivastava VC, Mall ID. An overview of various technologies for the treatment of dairy wastewaters. Critical reviews in food science and nutrition. 2011 Apr 14;51(5):442-52.
7. Al Hamed SG. Dairy wastewater treatment using microalgae in Karbala city–Iraq. IJEEFUS 4 (2014); 13. 2014;22.
8. Britz TJ, Van Schalkwyk C, Hung YT. Treatment of dairy processing wastewaters. Waste treatment in the food processing industry. 2006;1-28.
9. Ahmad T, Aadil RM, Ahmed H, ur Rahman U, Soares BC, Souza SL, Pimentel TC, Scudino H, Guimarães JT, Esmerino EA, Freitas MQ. Treatment and utilization of dairy industrial waste: A review. Trends in Food Science & Technology. 2019 Jun 1;88:361-72.
10. Woertz I, Feffer A, Lundquist T, Nelson Y. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. Journal of Environmental Engineering. 2009 Nov;135(11):1115-22.

11. Shete BS, Shinkar NP. Dairy industry wastewater sources, characteristics & its effects on environment. International Journal of Current Engineering and Technology. 2013 Dec;3(5):1611-5.

12. KolevSlavov A. General characteristics and treatment possibilities of dairy wastewater–a review. Food technology and biotechnology. 2017 Mar 23;55(1):14-28.

13. Gonçalves AL, Pires JC, Simões M. A review on the use of microalgal consortia for wastewater treatment. Algal Research. 2017 Jun 1;24:403-15.

14. Choi YK, Jang HM, Kan E. Microalgal biomass and lipid production on dairy effluent using a novel microalga, Chlorella sp. isolated from dairy wastewater. Biotechnology and Bioprocess Engineering. 2018 Jun;23(3):333-40.

15. Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environment international. 2013 Jan 1;51:59-72.

16. Hu J, Nagarajan D, Zhang Q, Chang JS, Lee DJ. Heterotrophic cultivation of microalgae for pigment production: A review. Biotechnology advances. 2018 Jan 1;36(1):54-67.

17. Bhalamurugan GL, Valerie O, Mark L. Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. Environmental Engineering Research. 2018;23(3):229-41.

18. Renuka N, Guldhe A, Prasanna R, Singh P, Bux F. Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. Biotechnology advances. 2018 Jul 1;36(4):1255-73.

19. Rodolfi L, ChiniZittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and bioengineering. 2009 Jan 1;102(1):100-12.

20. Wijffels RH, Barbosa MJ. An outlook on microalgal biofuels. Science. 2010 Aug 13;329(5993):796-9.

21. Msanne J, Xu D, Konda AR, Casas-Mollano JA, Awada T, Cahoon EB, Cerutti H. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae Chlamydomonas reinhardtii and Coccomyxa sp. C-169. Phytochemistry. 2012 Mar 1;75:50-9.

22. Abou-Shanab RA, Matter IA, Kim SN, Oh YK, Choi J, Jeon BH. Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake. biomass and bioenergy. 2011 Jul 1;35(7):3079-85.

23. Widjaja A, Chien CC, Ju YH. Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. Journal of the Taiwan Institute of Chemical Engineers. 2009 Jan 1;40(1):13-20.

24. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renewable and sustainable energy reviews. 2010 Jan 1;14(1):217-32.

25. Chen Z, Wang L, Qiu S, Ge S. Determination of microalgal lipid content and fatty acid for biofuel production. BioMed research international. 2018;2018.

26. Yao L, Gerde JA, Lee SL, Wang T, Harrata KA. Microalgae lipid characterization. Journal of agricultural and food chemistry. 2015 Feb 18;63(6):1773-87.

27. Harris EH. Chlamydomonas as a model organism. Annual review of plant biology. 2001 Jun;52(1):363-406.

28. Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Maréchal-Drouard L, Marshall WF. The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science. 2007 Oct 12;318(5848):245-50.

29. Grossman AR, Croft M, Gladyshev VN, Merchant SS, Posewitz MC, Prochnik S, Spalding MH. Novel metabolism in Chlamydomonas through the lens of genomics. Current opinion in plant biology. 2007 Apr 1;10(2):190-8.

30. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, Ruan RR. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresource technology. 2010 Apr 1;101(8):2623-8.

31. Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R. Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. Applied biochemistry and biotechnology. 2010 Oct;162(4):1174-86.

32. Dean AP, Sigee DC, Estrada B, Pittman JK. Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. Bioresource technology. 2010 Jun 1;101(12):4499-507.

33. Fan J, Andre C, Xu C. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in Chlamydomonas reinhardtii. FEBS letters. 2011 Jun 23;585(12):1985-91.

34. James SC, Boriah V. Modeling algae growth in an open-channel raceway. Journal of Computational Biology. 2010 Jul 1;17(7):895-906.

35. Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F, Triantaphylidès C, Li-Beisson Y, Peltier G. Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. BMC biotechnology. 2011 Dec;11(1):1-5.

36. Rani AS, Rao HG, Kumar AB, Shruthi M. Eco-Friendly Approach for Treating Dairy Effluent and Lipid Estimation Using Microalgae. Biotechnology Journal International. 2015 Apr 1:33-39.

37. Kothari R, Prasad R, Kumar V, Singh DP. Production of biodiesel from microalgae Chlamydomonas polypyrenoideum grown on dairy industry wastewater. Bioresource technology. 2013 Sep 1;144:499-503.

38. Ummalyma SB, Sukumaran RK. Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load. Bioresource technology. 2014 Aug 1;165:295-301.

39. Brar A, Kumar M, Pareek N. Comparative appraisal of biomass production, remediation, and bioenergy generation potential of microalgae in dairy wastewater. Frontiers in microbiology. 2019 Mar 29;10:678.

40. Hu X, Liu B, Deng Y, Bao X, Yang A, Zhou J. A novel two-stage culture strategy used to cultivate Chlorella vulgaris for increasing the lipid productivity. Separation and Purification Technology. 2019 Mar 18;211:816-22.

41. Morando-Grijalva CA, Vázquez-Larios AL, Alcántara-Hernández RJ, Ortega-Clemente LA, Robledo-Narváez PN. Isolation of a freshwater microalgae and its application for the treatment of wastewater and obtaining fatty acids from tilapia cultivation. Environmental Science and Pollution Research. 2020 Mar 24:1-10.

42. Federation WE, APH Association. Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA. 2005.

43. Hena S, Fatimah S, Tabassum S. Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resources and Industry. 2015 Jun 1;10:1-4.

44. Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresource technology. 2010 May 1;101(9):3097-105.

45. Liu C, Huang X, Wang X, Zhang X, Li G. Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. Phycologia. 2006 Mar 1;45(2):190-8.

46. Andersen RA, editor. Algal culturing techniques. Elsevier; 2005 Mar 4.